Pathogen profile

Beet poleroviruses: close friends or distant relatives?

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SUMMARY

Taxonomy: There are three members of the genus *Polerovirus* (family *Luteoviridae*) that induce yellowing of sugar beet: *Beet mild yellowing virus* (BMYV), *Beet chlorosis virus* (BChV) and *Beet western yellows virus*-USA (BWYV-USA, Fig. 1). Non-beet-infecting isolates of BWYV found particularly within Europe have now been re-named *Turnip yellows virus* (TuYV). Species-specific antibodies are unavailable, but the viruses can be distinguished by RT-PCR using primers specifically designed to the 5′ end of their respective genomes.

Physical properties: The isometric virus particles are approximately 26 nm in diameter and the genome consists of a single strand of positive sense RNA that utilizes almost all known plant virus gene expression strategies (initiation bypass, translational frameshifting and readthrough, synthesis of subgenomic RNA and proteolytic processing).

Host range: Many members of the *Chenopodiaceae* are susceptible, including commercial crops of sugar beet (*Beta vulgaris*), red beet and spinach. Experimental hosts include *Montia perfoliata*, *Nicotiana benthamiana* and *Arabidopsis thaliana*.

Symptoms: Sugar beet infected with beet poleroviruses show patches of chlorosis on the older leaves 4–6 weeks post-infection; these areas expand until the whole leaf becomes yellow and older leaves then tend to thicken and become brittle.

Transmission: Beet poleroviruses are transmitted in a persistent (circulative, non-propagative) manner by several different aphid species, *Myzus persicae* being the most important vector.

INTRODUCTION

The dissection of the relationships between the beet poleroviruses, as well as the clarification of their taxonomy, has been a goal for

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several decades. Problems began when *Beet mild yellowing virus* (BMYV) was described in the UK (Russell, 1958) almost at the same time as a similar virus was identified in North America. The American strains were initially called 'Radish yellows' but were subsequently re-named *Beet Western yellows virus* (BWYV) (Duffus, 1960). The host range of BMYV includes members of the *Chenopodiaceae* such as commercial crops of sugar beet and spinach. A number of weed species including *Capsella bursa-pastoris* and *Senecio vulgaris* are also experimentally and epidemiologically important hosts (Stevens *et al.*, 1994a). By contrast, American BWYV isolates cause stunting and chlorosis in a wider range of weed species and crop plants including sugar beet, spinach, lettuce and broccoli. In the 1970s, examination of UK weeds and crop plants, such as lettuce, showed that a BWYV-like virus was present in hosts previously reported as immune to BMYV (Duffus and Russell, 1970, 1972, 1975). These studies suggested the existence of a European isolate of 'BWYV' that is capable of infecting a wide range of hosts including crop plants in families other than the *Cruciferae*. Since these initial studies, the name 'BMYV' has been used by many to describe European isolates that are able to infect sugar beet, whereas European 'BWYV' isolates are regarded as infectious towards a broad range of commercially important *Brassica* crops (such as oilseed rape) and lettuce, but not sugar beet (Smith and Hinckes, 1985; Stevens *et al.*, 1994a). Of course, exceptions to this rule exist. For example, Polak (1979) isolated a virus from sugar beet that had a broad host range akin to the European non-beet-infecting strains of BWYV. Conversely, Lot and Maury-Chovelon (1985) found BWYV isolates from lettuce that were capable of infecting sugar beet. Unfortunately only host range and/or serological data are available for these examples and ideally sequence data would be required to compare and characterize these historical isolates in relation to current findings.

BEET CHLOROSIS VIRUS

To add to the beet polerovirus debate, another virus was identified in 1989 in both the USA (Duffus and Liu, 1991) and the UK (Stevens *et al.*, 1994b). In the UK, this newly discovered virus was originally
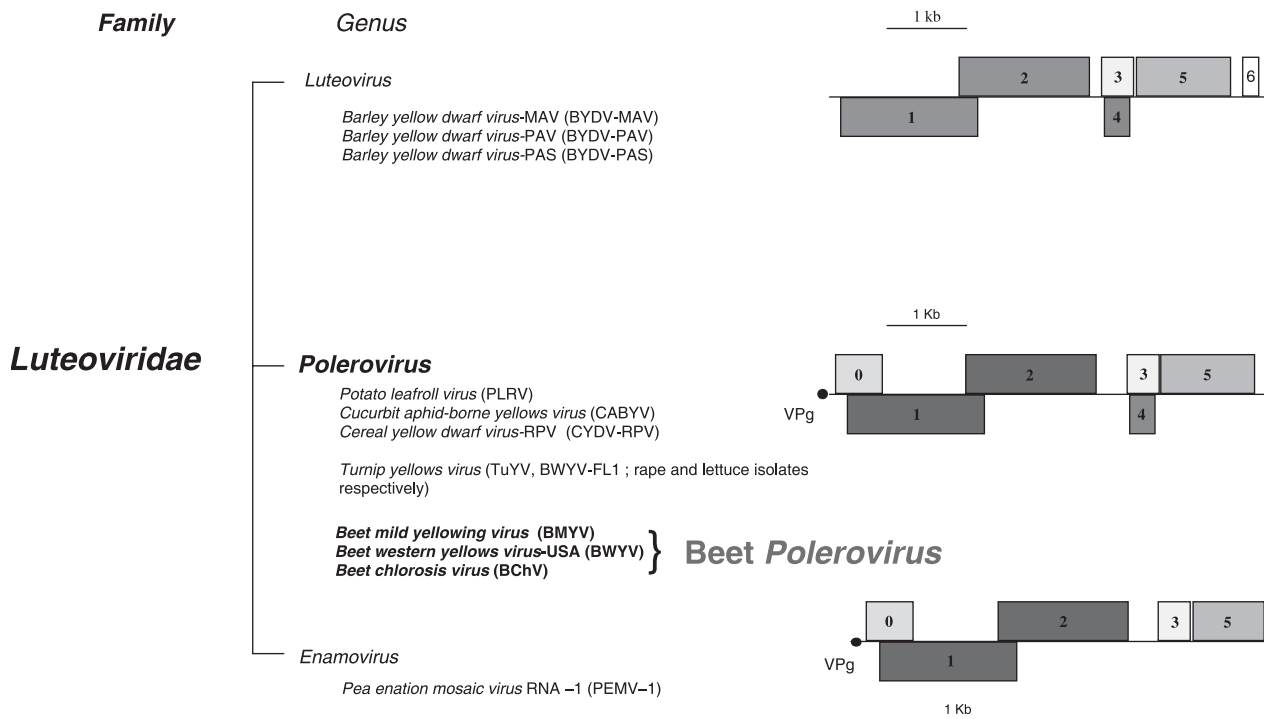
Fig. 1 Taxonomy and genome organization of members of the *Luteoviridae* family as adopted by ICTV (Mayo, 2002). The term 'Beet Polerovirus' indicates virus species (in bold type) that induce yellowing of sugar beet. Schematic diagrams of genome organization of the three *Luteoviridae* genera are shown.

Fig. 2 Symptoms of *Beet mild yellowing virus* (left) and *Beet chlorosis virus* (right) in sugar beet.

described as a second strain and serotype of BMYV because it produced paler symptoms in beet (Fig. 2), it failed to react with the key diagnostic monoclonal antibody BYDV-PAV-IL-1 (hence, similar to BWYV-USA and BWYV), and it did not infect the traditional indicator species *Montia perfoliata* and *C. bursa-pastoris*. In the USA, the new yellowing disease was found to be serologically related to BWYV, but also to exhibit a much narrower host range. This virus has frequently been observed in California,

Colorado, Nebraska and Texas sugar beet fields since the early 1990s (Duffus and Liu, 1991; Duffus *et al.*, 1999). Like the European isolates of BChV, the American strains also failed to infect *C. bursa-pastoris*. The name BChV was proposed due to the symptoms of interveinal chlorosis observed on infected sugar beet leaves in both the USA and Europe (Hauser *et al.*, 2002; Liu *et al.*, 1999). The virus has now been ratified by the International Committee for the Taxonomy of Viruses (ICTV) for inclusion as a new species in the genus *Polerovirus* (Mayo, 2002).

GENOMIC ORGANIZATION AND EXPRESSION

Viruses of the genus *Polerovirus* all share the same basic genome structure, and it is presumed that the expression strategy and gene function identified for one species will apply to all others within the genus (see previous reviews by Mayo and Miller, 1999; Mayo and Ziegler-Graff, 1996; Miller *et al.*, 1995). The BMYV genome (Fig. 3) consists of a linear, single-stranded plus sense RNA of *c.* 5.7 kb that is currently acknowledged to encode six open reading frames (ORFs).

The three 5′-proximal ORFs are expressed directly by translation from the genomic RNA (gRNA), while the ORFs downstream of a non-coding region (*c.* 200 nucleotides) are translated from a subgenomic RNA (sgRNA). The initiation of translation of ORF0 begins after a short leader sequence at the first AUG codon of the
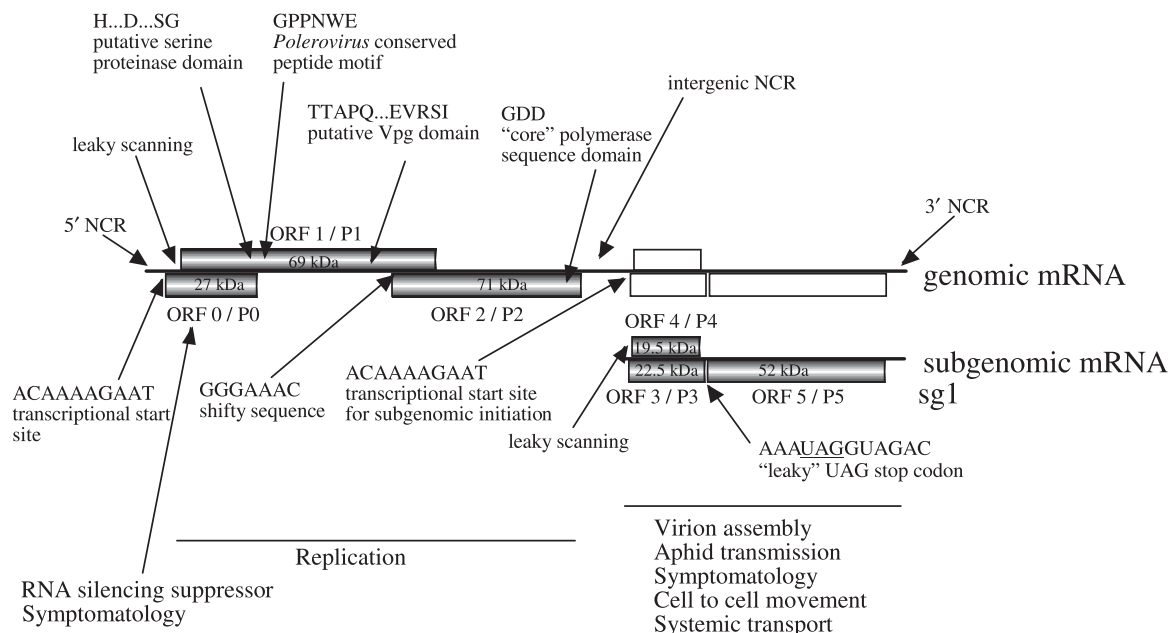
Fig. 3 Organization and expression of a representative beet polerovirus genome. The essential putative and deduced functions of the encoded proteins are indicated.

genome, but leaky scanning allows ribosomes to bypass this site in order to initiate translation at the start codon of ORF1. The ORF0 translation product has never been detected *in planta* although mutational analysis has demonstrated the importance of its expression for virus accumulation (Mayo and Ziegler-Graff, 1996; Sadowy *et al.*, 2001). The role of P0 has therefore been the subject of much speculation but recent evidence from work with a BWYV-lettuce isolate (syn. TuYV) (Pfeffer *et al.*, 2002) strongly implicates P0 as a suppressor of post-transcriptional gene silencing (PTGS) that would enable poleroviruses to overcome host resistance to infection (Waterhouse *et al.*, 2001).

The translation of ORF2 is achieved via a ribosomal frameshift from ORF1. Through mutational analysis, Reutenauer *et al.* (1993) have demonstrated that P1 and P2 are essential for infection, and both contain sequences strongly indicative of a role in replication (Mayo and Ziegler-Graff, 1996). P1 is known to contain protease motifs and also carries the amino acid sequence shown to be part of the viral genome-linked protein (VPg), which is found covalently associated with the 5′ end of the virus genome (van der Wilk *et al.*, 1997), while P2 carries the viral RNA-dependent RNA-polymerase (RdRp) domains harbouring the consensus core GDD motif (Mayo and Ziegler-Graff, 1996). As frameshifting is a rare event, it is predicted that P1 is expressed at greater levels than the P1 + P2 fusion (Miller *et al.*, 1995). Proteolytic processing of P1 or P1 + P2 polyprotein antecedents is postulated to result in the excision of the VPg (van der Wilk *et al.*, 1997), and may also yield further derivatives (Prüfer *et al.*, 1999).

Genes of the 3′-proximal cluster (ORFs 3, 4 and 5) are translated following the synthesis of sgRNA which is thought to depend on the initiation of the viral RdRp at internal promoter sites on the minus strand synthesized during gRNA replication. P3 is the major capsid protein. Mutational analyses using a BWYV-lettuce isolate have shown that the coat protein is not directly required for RNA replication in a protoplast system, although mutants unable to express ORF3 accumulated RNA to lower levels than the wild-type (Reutenauer *et al.*, 1993). This may be a consequence of the instability of the RNA progeny in the absence of encapsidation. Equally, it could indicate an as yet unknown regulatory role for P3 or an inhibitory (negative feedback) effect of unencapsidated RNA on replication (Mayo and Ziegler-Graff, 1996).

ORF5 is expressed as a consequence of translational read-through by suppression of the amber stop codon of ORF3 and P5 is therefore found only as a minor fusion protein (P3 + P5). The P5 read-through domain (RTD) has been shown through deletion and mutational analyses to exert no control over the initiation of infection or the assembly of virions (Reutenauer *et al.*, 1993). However, the protein is involved in symptom induction, virus accumulation and, potentially, in systemic spread (Brault *et al.*, 1995; Ziegler-Graff *et al.*, 1996), and plays a key role in transmission efficiency and specificity, as well as in virus persistence within the aphid vector (van den Heuvel *et al.*, 1997).

Similar to ORF1, ORF4 is translated by a leaky scanning mechanism. For all luteoviruses, excluding *Soybean dwarf virus* and BMYV, the ORF4 AUG start codon has a sequence context which is more favourable for the initiation of translation than that flanking the ORF3 initiation codon (Mayo and Ziegler-Graff, 1996). However, the exact *in vivo* ratio of P3 : P4 translation is unclear, although for PLRV it has been reported to be 1 : 1 (Juszczuk *et al.*,

2000). P4 expression is required for the systemic spread of virus infection in whole plants and may fulfil a putatively phloem-specific movement protein function.

A role for the structural proteins P3 and the P5 RTD in systemic movement have also been demonstrated (Bruyère *et al.*, 1997; Ziegler-Graff *et al.*, 1996). It has therefore been hypothesized that movement may be achieved via two parallel mechanisms. A movement protein-dependent pathway that facilitates the cell-to-cell transport of viral gRNA through plasmodesmata, and a P5-mediated means of virion translocation through sieve elements, are proposed (Ziegler-Graff *et al.*, 1996).

Work with a BWYV-lettuce isolate (Falk *et al.*, 1989) and, more recently, with PLRV and *Cucurbit aphid-borne yellows virus* (CABYV) (Ashoub *et al.*, 1998) has revealed the existence of a second subgenomic RNA. In BMYV, analysis of the full-length genome sequence published by Guilley *et al.* (1995) suggests that this may encode a single additional protein (P7) of as yet unknown function (Ashoub *et al.*, 1998).

DISCRIMINATION AMONG BEET POLEROVIRUSES

The discrimination and specific detection of beet poleroviruses is essential in order to study and clarify the role these viruses play in the epidemiology of virus yellows and associated disease complexes, as well as to ensure durable virus resistance in future resistant varieties. Polyclonal antisera raised to either BMYV or BWYV do not discriminate between the two poleroviruses (Casper, 1988; Govier, 1985) and attempts to produce BMYV-specific Mabs have been unsuccessful (Herrbach *et al.*, 1991; Smith *et al.*, 1996). The close serological relationship between BMYV and BWYV, and the similarities in host range, had led to claims that they were all strains of one virus (Hamilton *et al.*, 1981). Consequently, it had been proposed that only the name BWYV should be retained because of its widespread use within the literature (Casper, 1988). However, a monoclonal antibody originally raised against BYDV-PAV-IL can distinguish BMYV and BWYV (D'Arcy *et al.*, 1989) and it is now possible to identify these epitopes using coat protein structure models as were recently used to characterize the three-dimensional structure of the BWYV (syn. TuYV) capsid (Brault *et al.*, 2003). *Polerovirus* sequence diversity at the 5′ end of the genomes (Fig. 4) has been exploited to provide methods to discriminate between the beet poleroviruses (Lemaire *et al.*, 1995). Species-specific primers, used in multiplex RT-PCR, have now been developed to enable their detection in both plants and aphids (Hauser *et al.*, 2000a). Thus, the molecular characterization of the beet poleroviruses, together with their serological profiles and previous host range data, has clarified their relationships and resolved the nomenclature of these viruses. This has also helped to reveal the evolutionary pathways that gave rise to the *Luteoviridae*.

TAXONOMY AND POLEROVIRUS EVOLUTION

Recombination has been the key driving force behind the emergence of new beet poleroviruses as well as other species within the *Luteoviridae*; a family in which recombination has been described as 'rampant' (Gibbs, 1995). The general consensus is that the major point of recombination for these viruses is within the intergenic (non-coding) region. The genus *Polerovirus* is believed to have evolved from a recombination event between a sobemovirus and an ancestor that provided the 3′ properties (Gibbs, 1995; Martin *et al.*, 1990; Mayo and Ziegler-Graff, 1996). Molecular analysis of available beet polerovirus sequences confirms this view. Moreover, the low level of sequence homology at the 5′ end of the genomes (Fig. 4), alongside the biological and serological data, provides compelling evidence to support the fact that BMYV and the European non-beet-infecting strains of BWYV are actually two distinct virus species (Guilley *et al.*, 1995; Lemaire *et al.*, 1995). Initial sequence and phylogenetic analysis of the coat protein regions of geographically distinct beet polerovirus isolates has distinguished clusters within which the sequences are highly conserved (de Miranda *et al.*, 1995). Further analysis of the coat protein sequences of additional isolates showed that five clusters exist, corresponding to either their biological properties or geographical location (Hauser *et al.*, 2000b). Analysis of the P0 sequences (Hauser *et al.*, 2000b; Schubert *et al.*, 1998) enabled a clear distinction to be drawn between BWYV isolates infecting rape or lettuce and BMYV, thereby highlighting the existence of three distinct beet *Polerovirus* species. As a result, the ICTV has approved the re-classification of the non-beet-infecting strains of BWYV as a separate species in the genus *Polerovirus*, and these are now known as *Turnip yellows virus* (TuYV) (Mayo, 2002). Although other names had previously been proposed such as 'brassica yellowing virus' or 'lettuce yellows virus', TuYV was adopted because it had already been used in the literature as a synonym for European BWYV isolates (Graichen and Rabenstein, 1996; Van der Walle, 1950).

In the future, it is hoped that by adopting this new nomenclature for the beet poleroviruses, a clearer picture will emerge within the literature. It is also anticipated that it will remove the confusion that exists for applied virologists when attempting to describe precisely which virus species are present in the field. For example, the term BWYV is used in America to describe a wide range of isolates but this misleadingly encompasses strains with differing characteristics. For instance, a sugar beet infecting BWYV isolate from California has recently been sequenced and found to be more closely related to CABYV than BWYV as far as the replicase complex is concerned (M. Beuve, personal communication). Furthermore, alignment of the structural protein sequences show that the 3′ end of the virus has most identity with BMYV. Interestingly, the host range of this virus is more similar to BMYV than BChV or TuYV (syn. BWYV). Only by sequencing
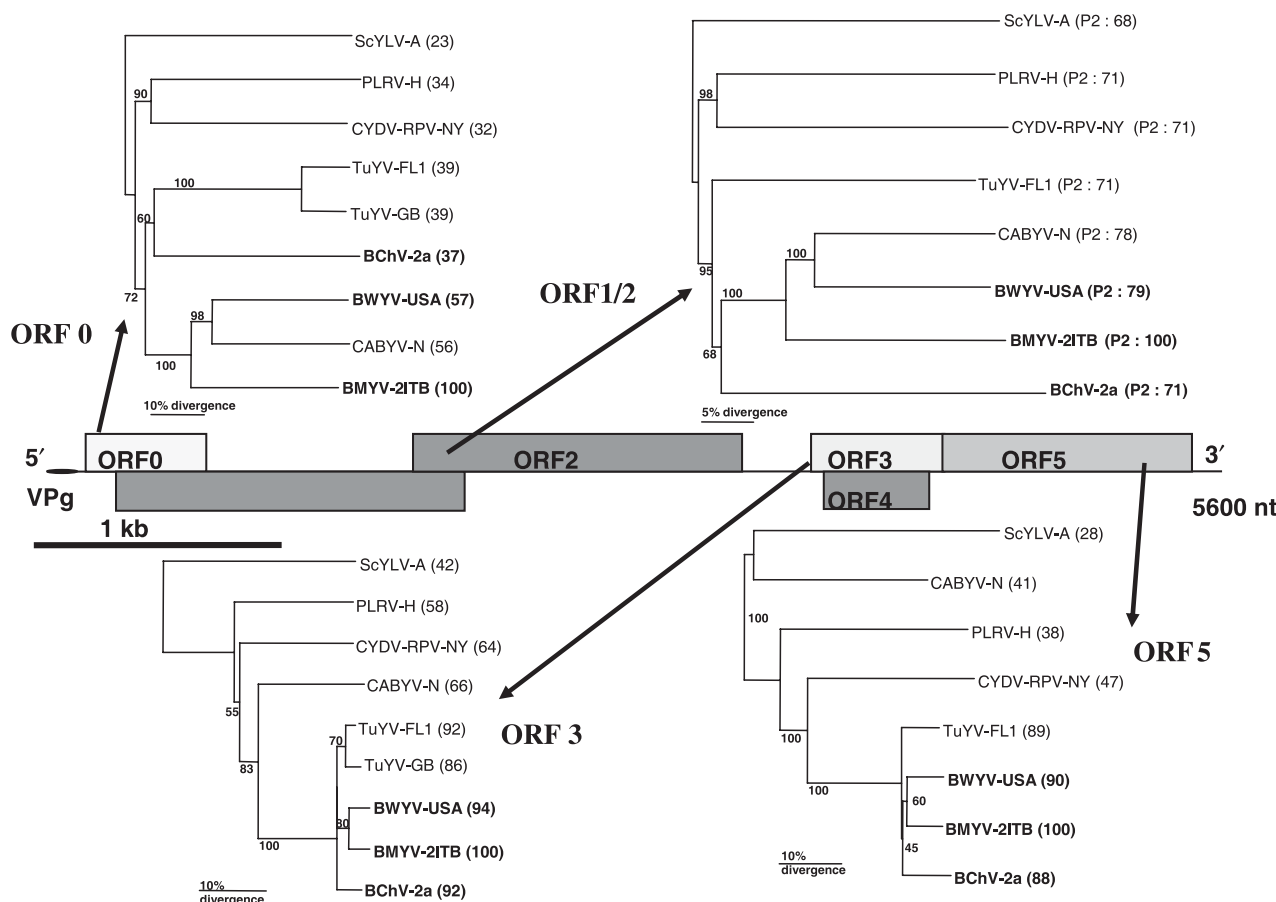
Fig. 4 Phylogenetic trees of P0, the polymerase complex (P1 + P2), major coat protein (P3) and read-through domain protein (P5) of various polerovirus genomes constructed using CLUSTALX. Bootstrap percentage values based on 1000 replicates are shown. Percentage protein similarities have been calculated using BMYV-2ITB as the reference species, and are indicated in brackets. Virus species inducing yellowing of sugar beet are in bold type. The strains used to draw these dendrograms are (acronym, GENBANK accession nos.): *Sugarcane yellow leaf virus* (ScYLV-A, AF157029), *Potato leafroll virus* (PLRV-H, Y07496), *Cereal yellow dwarf virus* (CYDV-RPV-NY, L25299), *Cucurbit aphid-borne yellows virus* (CABYV-N, X76931), *Beet mild yellowing virus* (BMYV-2ITB, X83110), *Beet chlorosis virus* (BChV-2a, AF352024), *Beet western yellows virus* (BWYV-USA-C, AF473561), *Turnip yellows virus* (TuYV-FL1, alias Beet western yellows virus-FL1, X13063, TuYV-GB, AF168608, AF167486).

and comparing a number of these American isolates will a clearer picture evolve and remove some of the confusion that has remained for a number of decades.

VIRUS-VECTOR RELATIONSHIPS

The plant-to-plant transmission of beet poleroviruses is obligately mediated by aphids (*Homoptera*, *Aphididae*) according to the circulative and non-propagative mode. The green peach aphid *Myzus persicae* (Sulzer), efficiently transmits all beet polerovirus species, whereas *Macrosiphum euphorbiae* (Thomas) is a less efficient vector of BMYV, BWYV-USA and TuYV, but surprisingly does not transmit British or American isolates of BChV (M.S., unpublished results). A few other species, such as *Myzus ascalonicus* Doncaster, *Aphis fabae* Scopoli and *A. gossypii* Glover, have been described as poor vectors (Heathcote, 1988; E.H., unpublished

results). However, these reports are likely to be related to specific virus strain–aphid clone combinations, due to the genetic variability of both partners (Gray and Gildow, 2003). Indeed, a systematic study of beet polerovirus vector specificity is lacking to date.

The route of beet polerovirus virions within the vector presumably resembles that observed for all other polerovirus species (Reinbold *et al.*, 2001; Taliansky *et al.*, 2003; for review see Gildow, 1999; Gray and Gildow, 2003; Reavy and Mayo, 2002). Virions are ingested from phloem tissues by the feeding aphid and acquired across the midgut wall. Particles then diffuse within the haemocoel and reach the accessory salivary glands, from whence they are released along with saliva. During this process, virions are transported across two epithelial layers, *viz.* the midgut and the accessory salivary glands, according to a receptor-mediated endocytosis/exocytosis mechanism. This mechanism is

thought to rely on specific interactions between viral capsid motifs and membrane-borne receptors, and to account for the narrow vector specificity.

The role of the RTD in virus–vector relationships seems to be complex; for BWYV (syn. TuYV), the viral motifs involved in transmission have been shown by mutational analyses to be borne by both the RTD (Brault *et al.*, 2001) and the coat protein (Brault *et al.*, 2003). RTD-lacking virions were found to be very inefficiently transported across the midgut wall, probably as a consequence of altered binding to putative membrane receptors or impaired transcytosis (Reinbold *et al.*, 2001). Moreover, when experimentally injected into the haemocoel, these virions were not observed near or in the accessory salivary glands, indicating that they are unable to enter the gland cells or are unstable in the haemocoel.

Thus far, less is known about the aphid components (receptors) involved in the specific recognition of the viral capsid. Recent biochemical studies on BWYV (syn. TuYV) identified several *M. persicae* polypeptides that, after 1- or 2-dimensional electrophoresis separation, are able to bind purified virions *in vitro* (Seddas *et al.*, 2004). Two of these polypeptides (of 35 and 37 kDa) are homologous to the *Drosophila melanogaster* proteins Rack-1 and GAPDH3. As RTD-lacking virions bind GAPDH3 but not Rack-1, it can be hypothesized that GAPDH3 is involved, as a coat protein (P3) receptor, in virus attachment to epithelial cells, whereas Rack-1 could enhance the efficiency of transcytosis of wild-type particles. Other clues to potential receptors arise from biochemical studies on the aphid *Sitobion avenae*, a vector of BYDV-PAV (the type member of genus *Luteovirus*). The comparison of two-dimensional electrophoretic patterns of a set of *S. avenae* clones revealed that several polypeptides correlated to the vectoring efficiency of clones (Papura *et al.*, 2002). However, whether these proteins act *in vivo* as virus receptors, or are only 'efficiency markers', remains unknown. Furthermore, polypeptides of 35 and 50 kDa extracted from *S. avenae* were proposed as potential receptors of BYDV-PAV and BYDV-GAV (Li *et al.*, 2001; Wang and Zhou, 2003). Future progress in the search for specific aphid receptors of beet poleroviruses and of other *Luteoviridae* members will benefit from increasing knowledge in aphid genomics and proteomics. Elucidating the nature and role of these receptors may aid the development of novel means for controlling virus spread by disrupting virion–vector interactions.

A further striking feature of BWYV (syn. TuYV) and other *Luteoviridae* members is their ability to bind a protein named symbionin. This is a homologue of *Escherichia coli* GroEL, which is produced in the aphid by endosymbiotic bacteria of the genus *Buchnera* (van den Heuvel, 1999). Symbionin is known to bind the RTD *in vitro*, which may account for the observed instability of RTD-lacking virions in the aphid haemocoel. This interaction is thought to protect the particles from proteolysis by haemolymph factors, and/or to prepare the virions to enter the accessory salivary gland cells. More generally, the understanding of how the virus escapes or overcomes the defence systems of the vector represents an as yet unexplored but challenging area of research. Gildow (1999) hypothesized that species of the *Luteoviridae* had evolved from an entomopathogenic ancestor. Co-evolution of the virus–insect–plant trinomial may have driven the virus to acquire an ability to replicate in phloem tissues, then to progressively lose its aggressivity toward the insect and eventually to become unable to replicate in the latter. In the course of this evolution, the virus presumably adapted to the innate immune response of its insect host. Recent work on thrips (*Thysanoptera*), which are vectors of *Tomato spotted wilt virus* (TSWV, *Tospovirus*) according to a persistent and (moderately) propagative mode of interaction, revealed that immunity-related proteins, such as Toll-3 receptor and antimicrobial peptides, are overexpressed in viruliferous vectors. This suggests that TSWV infection activates the immune system of the insect (Medeiros *et al.*, 2004). It is envisaged that a greater understanding of the biochemical mechanisms underlying the intimate association between virus particles, aphid cells and phloem factors may lead to novel control approaches against economically important crop diseases caused by viruses of the *Luteoviridae*, including beet poleroviruses.

RESISTANCE

Early infection of sugar beet with BMYV can decrease sugar yield by up to 29% (Smith and Hallsworth, 1990) and affects the extractability of the sugar by the processors. BChV is less damaging than BMYV when plants are infected early in the growing season, but if plants become infected later in the summer, then BChV causes a greater yield loss than BMYV (Stevens *et al.*, 2004). Resistant sugar beet cultivars would provide an alternative, environmentally acceptable control strategy but, to date, no such varieties have been developed. No major sources of BMYV resistance have been identified, though partial resistance has been found in other species of the genus *Beta* (Asher *et al.* 2001). Of the 22 accessions that were identified as being partially resistant to BMYV (out of 600 screened) only three lines showed additional resistance to BChV. A major quantitative trait locus has been found for resistance to TuYV in winter oilseed rape enabling marker-assisted selection for virus resistance (Dreyer *et al.*, 2001). Identification of major gene sources of resistance to the beet infecting poleroviruses would be a major breakthrough and would remove the need for widespread, prophylactic use of insecticide treatments. In the USA a number of sugar beet lines have been developed at USDA-Salinas, California that have tolerance/partial resistance to BWYV. More recently, BChV resistance has been identified (Lewellen *et al.*, 1999), although the inheritance of this trait is as yet unknown.

Fig. 5 *Arabidopsis thaliana* Col-0 infected with BMYV.

CONCLUSIONS

Since their initial identification and characterization, the beet poleroviruses have been found ubiquitously and demonstrated to cause significant damage in sugar beet and other crops worldwide. However, the spectrum of isolates found in the field has resulted in much confusion both within the literature and in particular for extension virologists when attempting to identify the causal agent of a specific problem. On the basis of current evidence, three closely related but distinct beet polerovirus species have been defined from the continuum of biologically different strains. The comparison of 5′-proximal regions from all available polerovirus genome sequences neatly places isolates into three distinct groups that correlate to their host specificity in sugar beet, oilseed rape or *C. bursa-pastoris*. Further work is required to determine whether ORF0 and/or ORF1 contain the host range determinants of these viruses. We have recently developed a full-length infectious cDNA clone of BMYV and intend to undertake mutational analysis and gene exchange experiments with BChV and TuYV to pin-point the factors controlling pathogenicity and host range.

Although BChV has been accepted as a new species within the genus *Polerovirus*, little is presently known about the world-wide distribution of this virus or its vector specificity and why the host range appears to be limited to *Beta* species and the weed *Chenopodium capitatum*. Having such a narrow ecological niche might draw many to the conclusion that this virus has little economic or agricultural significance. However, recent studies have in fact shown that BChV is gaining in importance both in Europe and the USA (Stevens *et al.* in press). Preliminary studies in sugar beet have demonstrated that the effects of co-infection with BChV and BMYV are not cumulative, implying that cross-protection may occur, as has been previously reported between strains of BYDV (Wen *et al.*, 1991). The molecular mechanisms behind these observations are currently being investigated.

We have recently identified *Arabidopsis thaliana* as a host for BMYV and TuYV (Fig. 5), but all ecotypes screened to date have proved resistant to BChV. These findings provide a valuable model pathosystem with which to study vector–virus–host interactions such as gene silencing phenomena (both pathogen-derived and virus-induced) and gene regulation in a host/non-host environment. It is ultimately hoped that naturally occurring or engineered resistance, essentially to all beet poleroviruses, may be exploitable to replace the current use of insecticides and provide durable and environmentally acceptable control strategies for the future.

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